Histological distinction between immature and regenerating females and its effect on maturity ogive estimation in three tropical hermaphroditic groupers

by

Thierry BRULÉ* & Teresa COLÁS-MARRUFO (1)

Abstract. – Accuracy of the muscle bundle criterion is analysed to distinguish regenerating ovaries from immature ones in three commercially important hermaphroditic grouper species from the southern Gulf of Mexico: red grouper *Epinephelus morio* (Valenciennes, 1828); black grouper *Mycteroperca bonaci* (Poey, 1860); and gag *Mycteroperca microlepis* (Goode & Bean, 1880). The presence of muscle bundles in ovaries is a generally accurate criterion for distinguishing between regenerating and immature females in these species. However, reproductively inactive specimens could not be exactly identified as regenerating or immature females in 22% of *E. morio*, 4% of *M. bonaci* and 9% of *M. microlepis*, and are classified as being of uncertain maturity. Median size and size-frequency distributions analysis among females in different reproductive phases indicate that uncertain maturity *E. morio* females are probably immature, while uncertain maturity *M. bonaci* females are probably regenerating. The reproductive status of uncertain maturity *M. microlepis* females could not be clarified. Maturity ogive and size at maturity estimates for females of the studied species exhibit variable bias in response to the percentage of uncertain maturity females.

© SFI
Received: 1 Apr. 2012
Accepted: 19 Apr. 2013
Editor: O. Otero

Résumé. – Distinction histologique entre femelles immatures et en régénération, et ses conséquences sur l'estimation des ogives de maturité chez trois mérous tropicaux hermaphrodites.

L'intérêt de la présence de paquets musculaires dans les ovaires de poisson, afin de distinguer les femelles en régénération de celles immatures, a été évalué chez trois mérous de valeur commerciale du golfe du Mexique : le mérou rouge *Epinephelus morio* (Valenciennes, 1828), la badèche bonaci *Mycteroperca bonaci* (Poey, 1860) et la badèche baillou *Mycteroperca microlepis* (Goode & Bean, 1880). Pour ces trois espèces, ces paquets musculaires représentent un critère microscopique fiable pour la distinction des femelles en régénération et des femelles immatures. Toutefois, 22% des femelles sexuellement inactives d'*E. morio*, 4% de *M. bonaci* et 9% de *M. microlepis* ne purent être classifiées ni en régénération ni comme immature et sont alors considérées en phase de maturité incertaine. Les tailles médianes et les distributions de fréquence de taille de ces individus indiquent que les femelles d'*E. morio* en maturité incertaine sont probablement des individus immatures, tandis que celles de *M. bonaci* sont probablement des individus en régénération. Pour *M. microlepis* il ne fut pas possible de confirmer le statut sexuel de ces femelles. Pour les trois espèces, l'estimation des ogives de maturité et de la taille de première maturité sexuelle présente des biais variables en relation avec le pourcentage de femelles classifiées en maturité incertaine.

Key words
Serranidae
Epinephelus morio
Mycteroperca bonaci
Mycteroperca microlepis
Grouper
Ovary histology
Reproductive phases
Length at maturity

Reliable identification of reproductive phases in fish gonads is essential to determining sexual pattern, sexual cycle, and size or age of sexual maturation. Data on maturity is incorporated into stock assessment by means of maturity ogive estimation in calculation of spawning stock biomass (SSB). Information on size at maturity can also be used to develop minimum landing size regulations (Morgan, 2008). Maturity ogives are usually estimated by macroscopic examination of gonads, but this can lead to drastic misclassification of fish reproductive status, and consequently to underestimates (ICES, 2008) or overestimates (Vitale *et al.*, 2006) of the proportion of mature individuals. Indeed, distinguishing between regenerating (e.g. sexually mature but reproductively inactive; Brown-Peterson *et al.*, 2011) and immature (virgin and reproductively inactive) females can be extremely difficult when done by macroscopic inspection of ovaries. Significant misclassification of regenerating females as immature females compromises maturity data quality, resulting in SSB underestimates and erroneous management decisions (ICES, 2008). In hermaphroditic fish species, macroscopic distinction cannot be accurately made between immature and regenerating ovaries, and between these ovary stages and immature, regressing and regenerating testes. Histological analysis of gonad development is therefore the only reliable method for determining female reproductive phase in these species.

Histological examination of ovaries may provide the most accurate way of identifying mature inactive individuals, but characterization of regenerating females still poses a challenge because their ovaries contain the same oocyte stag-

(1) Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Departamento de Recursos del Mar, Unidad Mérida, Antigua Carretera a Progreso Km. 6, 97310 Mérida, Yucatán, México. [tcolas@mda.cinvestav.mx]
* Corresponding author [tbrule@mda.cinvestav.mx]

es [e.g. oogonia and primary growth (PG) oocytes] as immature females (Rideout *et al.*, 2005). Regenerating phase ovaries can be differentiated from immature phase ovaries based on the presence of six traits: (1) circumnuclear oil droplets in PG oocytes; (2) thicker ovarian wall; (3) more interstitial tissue and capillaries surrounding the PG oocytes; (5) late-stage atresia and muscle bundles; and (6) degenerating post-ovulatory follicles (POFs) (Brown-Peterson *et al.*, 2011). The type and number of criteria used to identify regenerating females varies between species. Some criteria sets can be more complicated to implement than others. For example, some overlap may exist in wall thickness between immature and regenerating ovaries, and POFs may be present only briefly due to rapid degeneration and are only useful while they persist in the ovary after spawning (Rideout *et al.*, 2005). In a number of species, particularly groupers, the presence of muscle bundles in the ovaries of regenerating females is considered a reliable sign of prior spawning activity and is generally seen for a number of months after spawning (Shapiro *et al*, 1993; Sadovy *et al.*, 1994; McGovern *et al.*, 1998; Rhodes and Sadovy, 2002; Brown-Peterson *et al.*, 2011).

Tropical grouper species have a set of intrinsic reproductive features which make them more vulnerable to fishing impact: they have late sexual maturity; are generally protogynous hermaphrodites; and several species aggregate to spawn (Coleman *et al.*, 2000; Levin and Grimes, 2002). In the southern Gulf of Mexico, the grouper fishing stocks from the Yucatán Peninsula continental platform (Campeche Bank, Mexico) are fully exploited (SAGARPA, 2012); indeed, red grouper *Epinephelus morio* (Valenciennes, 1828) is overexploited in this region (Burgos and Defeo, 2004). At least 18 grouper species are commercially exploited on the Campeche Bank. Based on catch number and weight, the most commercially important species are *E. morio*, followed by black grouper, *Mycteroperca bonaci* (Poey, 1860) and gag, *Mycteroperca microlepis* (Goode & Bean, 1880) (Colás-Marrufo *et al.*, 1998; Brulé *et al.*, 2009). All three species are protogynous hermaphrodites, and *M. bonaci* and *M. microlepis* are aggregating spawners (Domeier and Colin, 1997; Brulé *et al.*, 1999, 2003a, 2003b).

The present study objective is to test the accuracy of muscle bundle criteria in distinguishing regenerating from immature ovaries in *E. morio*, *M. bonaci* and *M. microlepis* from the Campeche Bank. These data will help to develop more accurate tools to assess maturity phase and size at maturity, and increase management efficiency for the grouper fishery and stocks in this region.

MATERIAL AND METHODS

Epinephelus morio, *Mycteroperca bonaci* and *M. microlepis* are depth-size distributed over their geographical range: smaller individuals are common inshore while larger ones inhabit deeper waters. To collect an accurate size profile, specimens were collected from commercial catches taken from rocky and coral bottoms, in offshore waters (30-210 m depth) and in coastal inshore waters (1-27 m depth) of the Campeche Bank. Monthly samples were randomly obtained from commercial catches, meaning specimens had to be collected during several successive years to complete a single year study including reproductive and nonreproductive seasons for each species. *Epinephelus morio* specimens were caught between August 1988 and September 1993; *M. bonaci* specimens between April 1996 and February 2000; and *M. microlepis* specimens between April 1996 and December 2001. Fishing gear included long-lines for offshore catches and hook-and-line, spear guns or trawl nets for inshore and coral reef catches.

Body length at the caudal fork (FL) was recorded to the nearest 0.1 cm, and gonad tissues from each collected specimen processed for light microscopy. For each specimen, a sample was taken from the central mid-section of one gonad and fixed in Bouin's fluid (Gabe, 1968). Gonad samples were stored separately in tissue processing embedding cassettes, dehydrated with an ethanol series, "cleared" with toluene and infiltrated in melted Paraplast tissue embedding medium heated to 60°C (pellet melting point = 56°C). The infiltrated gonad samples were placed in stainless steel base moulds and embedded in Paraplast. Sections (6 μm) of gonad tissue in Paraplast were taken with a semi-automatic rotary microtome. These sections were stretched with warm (38-40°C) water in a flotation bath, mounted on slides and placed in an oven (37°C) overnight. The gonad sections were stained manually following Gabe and Martoja one-step trichrome procedure (Gabe, 1968), mounted with Canada balsam natural resin and placed in an oven for 48 h to 72 h at 37°C.

In each species, females were identified and selected by examination of histological sections of gonads. Of the histologically assessed individuals, females accounted for 80% (N = 812) of *E. morio* (total N = 1022), 82% (N = 1020) of *M. bonaci* (total N = 1250) and 84% (N = 268) of *M. microlepis* (total N = 319).

Histological description of female germ cells (stages) was done based on Wallace and Selman (1981) and Lowerre-Barbieri *et al.* (2009). Reproductive phase classification follows the terminology proposed by Brown-Peterson *et al.* (2011): immature; developing; spawning capable (with actively spawning sub-phase); regressing; and regenerating (Tab. I). Mature females (i.e. adult; Brown-Peterson *et al.*, 2011) were classified either as mature active (developing, spawning capable, actively spawning and regressing phase or sub-phase) or as mature inactive when regenerating. Histologically, regenerating females are distinguished from immature (virgin) females (no previous spawn) by the presence of muscle bundles in ovaries, which are defined as

Table I. - Histological criteria used to determine reproductive phases in *Epinephelus morio*, *Mycteroperca bonaci* and *Mycteroperca microlepis* females from Campeche Bank, southern Gulf of Mexico (from Lowerre-Barbieri *et al.*, 2009; Brown-Peterson *et al.*, 2011).

Reproductive phase	Histological features
Immature	Only oogonia (OG) and primary growth (PG) oocytes present.
Developing	OG, PG and cortical alveolar (CA), primary and secondary vitellogenic (Vtg1 and Vtg 2) oocytes present.
Spawning capable	OG, PG, CA, Vtg1, Vtg2 and tertiary vitellogenic (Vtg3) oocytes present. Oocytes in early stage of maturation (OEM), i.e. early stage of germinal vesicle migration (GVM), can be present. *Actively spawning subphase*: OG, PG, CA, Vtg1, Vtg2, Vtg3 and oocytes in late stages of maturation (OLM), i.e. completed GVM or germinal vesicle breakdown (GVBD) with yolk coalescence and hydration, present. Post-ovulatory follicles (POFs) can be present.
Regressing	OG, PG, and some residual CA, Vtg1, Vtg2, and Vtg3 oocytes present, most of them in atresia. POFs can be present.
Regenerating	Only OG and PG oocytes present with muscle bundles, connective tissue and blood vessels present at the center of ovarian lamellae.

Table II. - Comparison of median sizes and size-frequency distributions between females in immature (I), regenerating (R), mature active (MA) and uncertain maturity (UM) phases for *Epinephelus morio*, *Mycteroperca bonaci* and *Mycteroperca microlepis* collected on Campeche Bank, southern Gulf of Mexico. For a given species and sampling location, median sizes (Mann-Whitney or Kruskal-Wallis test) and size ranges (Kolmogorov-Smirnov test) with the same lowercase letter are not significantly different.

Species	Sampling location	Reproductive phase	Fork length (cm)		
			Median	Rango	N
Epinephelus morio	Inshore	I	26.3^{a}	$17.5\text{-}54.5^{w}$	122
		UM	35.5^{a}	$15.0\text{-}55.5^{w}$	16
	Offshore	I	54.1^{a}	$44.5\text{-}69.0^{w}$	112
		R	61.0^{b}	$42.2\text{-}83.5^{x}$	182
		MA	61.3^{b}	$38.9\text{-}85.4^{x}$	277
		UM	55.5^{a}	$44.5\text{-}82.3^{w}$	103
Mycteroperca bonaci	Inshore	I	41.0	25.6-58.0	38
	Offshore	I	62.0^{a}	$44.0\text{-}88.0^{w}$	280
		R	101.0^{b}	$64.0\text{-}123.0^{x}$	367
		MA	93.0^{c}	$58.0\text{-}123.5^{y}$	309
		UM	92.3^{c}	$54.4\text{-}117.0^{z}$	26
Mycteroperca microlepis	Inshore	I	26.6	9.3-49.2	107
	Offshore	I	74.0^{a}	$49.5\text{-}87.5^{w}$	13
		R	94.0^{b}	$70.5\text{-}109.0^{x}$	71
		MA	95.0^{b}	$72.0\text{-}105.0^{x}$	59
		UM	84.6^{c}	$74.5\text{-}111.0^{y}$	18

blood vessels surrounded by muscle and connective tissue (Shapiro *et al.*, 1993). Specimens that did not clearly classify into one of these phases were assigned to an uncertain maturity phase (Lyon *et al.*, 2008).

Comparisons were made of median FL and FL-frequency distributions for immature, regenerating and mature active females for each grouper species to ensure that specimens were correctly assigned to the immature or regenerating female phases (Sadovy *et al.*, 1994; McGovern *et al.*, 1998; Harris *et al.*, 2002; Rhodes and Sadovy, 2002). The median FL and FL-frequency distributions of females in the uncertain maturity phase were compared to those of immature, regenerating and mature active individuals to determine if they could be considered either immature or regenerating. Fish were grouped into 15 cm (*E. morio*) and 21 cm (*M. bonaci*; *M. microlepis*) FL classes at a 5 cm interval following Sturges method (Scherrer, 1984). A Mann-Whitney test (when analysed reproductive phases = 2; for *E. morio* inshore collection) or a Kruskal-Wallis test (when analysed reproductive phases > 2; for *E. morio*, *M. bonaci* and *M. microlepis* offshore collections) were applied to compare median FL. A Kolmogorov-Smirnov nonparametric test was used to compare FL-frequency distributions. Nonparametric statistics were used because mean and standard deviation based on normal theory did not apply.

Using the reproductive phases for the three grouper species as identified by histological examination of ovaries, the maturity ogives and lengths at which 50% of females were

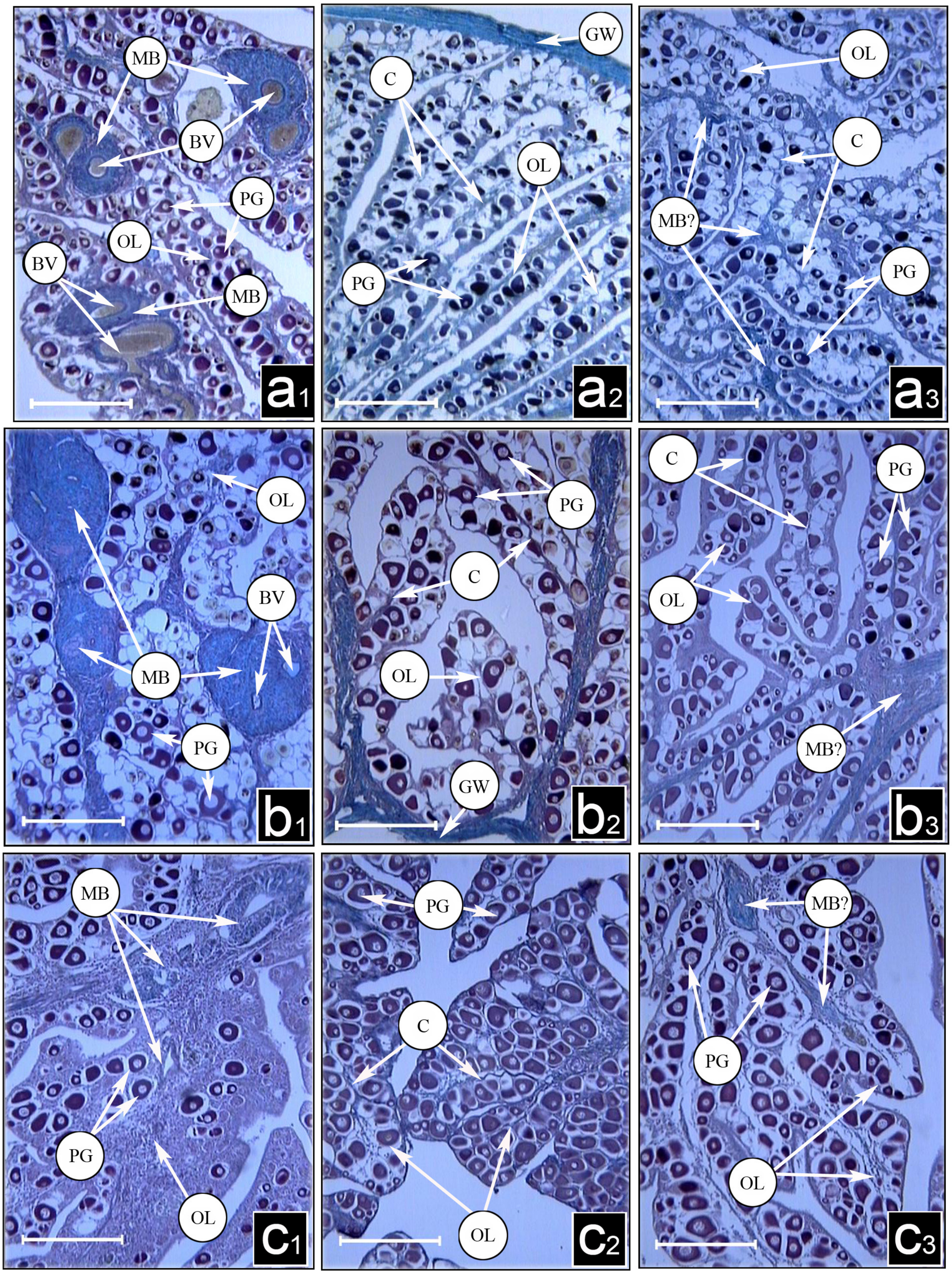

Figure 1. - Histological sections of ovaries in female *Epinephelus morio* (a), *Mycteroperca bonaci* (b) and *Mycteroperca microlepis* (c) in the regenerating phase (1), immature phase (2) and uncertain maturity phase (3). a_1: *E. morio*, 72 cm FL, collected Jun. 1990; a_2: *E. morio*, 68 cm FL, collected Jun. 1989; a_3: *E. morio*, 51 cm FL, collected Aug. 1989. b_1: *M. bonaci*, 107 cm FL, collected Jul. 1997; b_2: *M. bonaci*, 62 cm FL, collected Oct. 1997; b_3: *M. bonaci*, 65 cm FL, collected Jun. 1998. c_1: *M. microlepis*, 97 cm FL, collected Jul. 1998; c_2: *M. microlepis*, 82 cm FL, collected Aug. 1997; c_3: *M. microlepis*, 89 cm FL, collected Jul. 1998. C, cord; BV, blood vessel; GW, gonad wall; MB, muscle bundle; OL, ovarian lamellae; PG, primary growth oocyte. Gabe and Martoja one-step trichrome stain. Scale bars = 300 microns.

sexually mature (L_{50}) were calculated using a logistic regression model ($L_{50} = e^z (1 + e^z)^{-1}$, where $z = a + b \times \log_{10}L$, with L = FL) fitted to the data using the maximum likelihood method.

Statistical calculations were done using the SYSTAT 8.0 (www.systat.com) and INFOSTAT (www.infostat.com.ar) programs. All statistical analyses were run with $\alpha = 0.05$ significance level.

RESULTS

Ovary histology

Most of the females collected during the study are reproductively inactive since their ovaries contain only oogonia and PG oocytes (Tab. II; 66% *E. morio*; 70% *M. bonaci and* 78% *M. microlepis*). Of these individuals, typical muscle bundles are observed in the ovaries of 34% of *E. morio*, 51% of *M. bonaci* and 34% of *M. microlepis*. When present, muscle bundles are well-developed, abundant and always located in the centre region of the ovarian lamellae (Fig. $1a_1$, b_1, c_1). Individuals with these characteristics are classified as regenerating females (Tab. II). When no muscle bundles are observed in the ovaries of reproductively inactive females (44% of *E. morio*, 45%, of *M. bonaci* and 57% of *M. microlepis*), they were classified as immature (Fig. $1a_2$, b_2, c_2; Tab. II). The remaining reproductively inactive females (22% of *E. morio*, 4% of *M. bonaci*, 9% *M. microlepis*) exhibit some small connective tissue structures scattered in the centre of the ovarian lamellae (Fig. $1a_3$, b_3, c_3). Some doubt remains as to the nature of these structures. As a result, these specimens could not be definitively assigned to an immature or regenerating phase and are classified as being in the uncertain maturity phase (Tab. II).

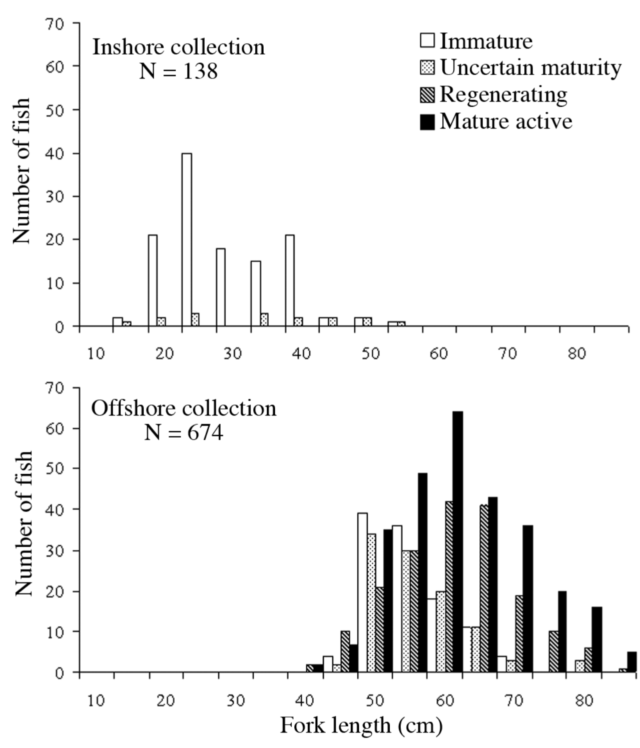

Figure 2. - Size-frequency distributions of *Epinephelus morio* females in immature, uncertain maturity, regenerating and mature active (developing, spawning capable, actively spawning and regressing) phases, collected in inshore and offshore waters of Campeche Bank, southern Gulf of Mexico.

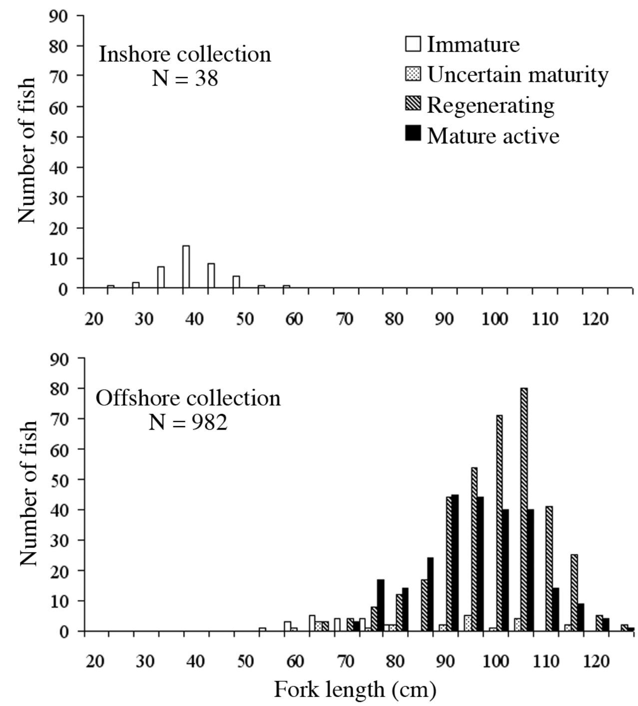

Figure 3.- Size-frequency distributions of *Mycteroperca bonaci* females in immature, uncertain maturity, regenerating and mature active (developing, spawning capable, actively spawning and regressing) phases, collected in inshore, offshore waters and coral reefs of Campeche Bank, southern Gulf of Mexico.

Size-frequency distributions

Females from all three grouper species caught in inshore waters are identified as immature, except for a small number of *E. morio* (N = 16) classified as of uncertain maturity (Figs 2-4; Tab. II). Median FL and FL-frequency distributions for these *E. morio* females do not differ significantly from those of immature *E. morio* females (Tab. II). This suggests that the *E. morio* females classified in the uncertain maturity phase are probably all immature specimens.

Offshore specimens of all three species include immature, regenerating, mature active and uncertain maturity females (Figs 2-4). The median FL and FL-frequency distribution for regenerating *E. morio* and *M. microlepis* females do not differ significantly from those of mature active ones.

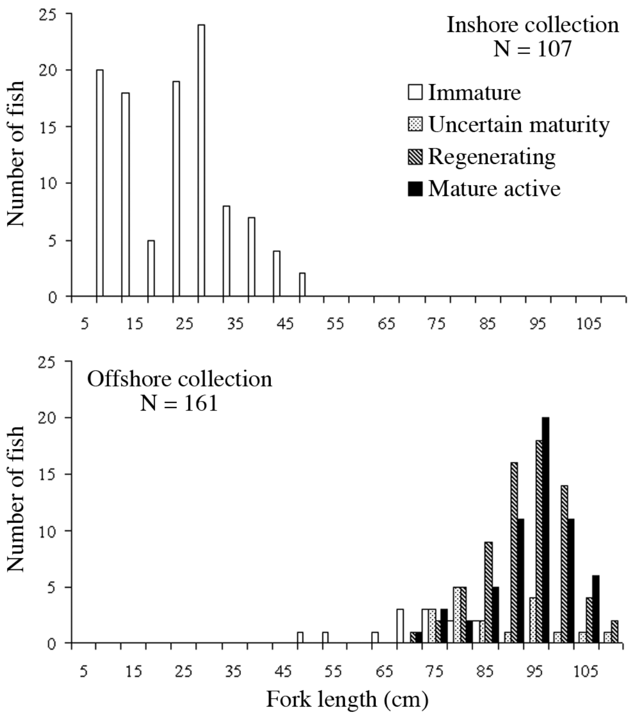

Figure 4. - Size-frequency distributions of *Mycteroperca microlepis* females in immature, uncertain maturity, regenerating and mature active (developing, spawning capable, actively spawning and regressing) phases, collected in inshore and offshore waters of Campeche Bank, southern Gulf of Mexico.

Conversely, the median FL and FL-frequency distribution for regenerating *M. bonaci* females are higher than for mature active ones (Tab. II). These results indicate that females with visible muscle bundles in the ovaries were correctly assigned to the regenerating reproductive phase.

Median FL and FL-frequency distribution for female *E. morio* in the uncertain maturity phase do not differ significantly from those of immature individuals (Tab. II). These can therefore probably be assigned to the immature phase. For *M. bonaci* females, the median size and size-frequency distribution of uncertain maturity individuals do not differ significantly from those of mature active females (Tab. II). This implies that these females are probably regenerating individuals. Finally, median FL and FL-frequency distribution for uncertain maturity *M. microlepis* females differ significantly from those of individuals in any other identified phase (Tab. II). It was therefore not possible to gener-

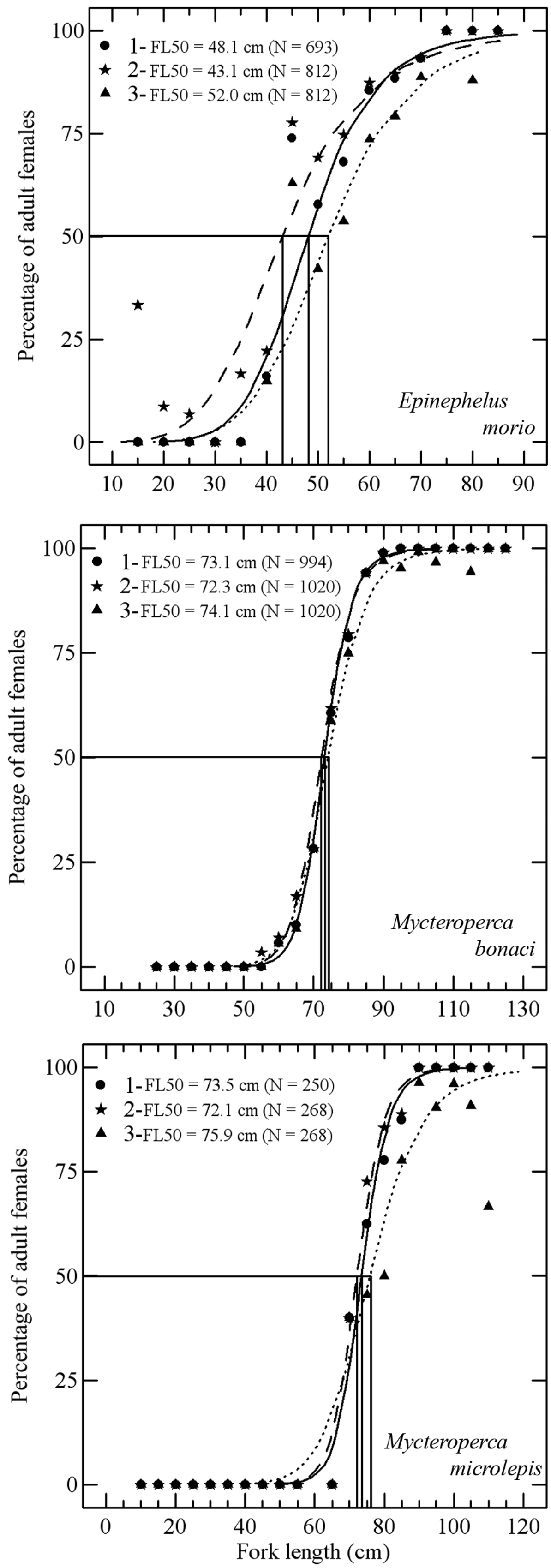

Figure 5 (right). - Maturity ogives and L_{50} (length at which 50% of females are mature) estimates for *Epinephelus morio*, *Mycteroperca bonaci* and *Mycteroperca microlepis* females from Campeche Bank, southern Gulf of Mexico. Maturity ogives: 1(●), uncertain maturity females excluded; 2(★), uncertain maturity females included as regenerating; and 3(▲), uncertain maturity females included as immature. The proportion of adult females within each size-class is fitted using a logistic function and maximum-likelihood method.

ate a more accurate definition of reproductive status for the *M. microlepis* uncertain maturity females.

Maturity ogives and length at maturity

Ovaries in the uncertain maturity phase were identified in each of the three grouper species, so three maturity curves were generated per species (Fig. 5). The first curve does not include uncertain maturity females (Fig. 5: 1); the second includes them as regenerating females (Fig. 5: 2); and the third includes them as immature females (Fig. 5: 3). In all three species, the L_{50} values calculated from the maturity ogives decrease when uncertain maturity females are treated as regenerating individuals (Fig. 5: 2), but increase when they are treated as immature individuals (Fig. 5: 3). The difference between the calculated minimum and maximum L_{50} values are higher for *E. morio* (δ = 8.9 cm) than for *M. microlepis* (δ = 3.8 cm) and *M. bonaci* (δ = 1.8 cm) (Fig. 5).

DISCUSSION

Prominent muscle bundles have been reported previously in the ovaries of regenerating females of different grouper species such as red hind, *Epinephelus guttatus* (Linnaeus, 1758) (Shapiro *et al*., 1993; Sadovy *et al*., 1994); camouflage grouper, *Epinephelus polyphekadion* (Bleecker, 1849) (Rhodes and Sadovy, 2002); Nassau grouper, *Epinephelus striatus* (Bloch, 1792) (Carter *et al*., 1994); scamp, *Mycteroperca phenax* (Jordan & Swain, 1885) (Harris *et al*., 2002) and *M. microlepis* from the Atlantic coast of the south-eastern United States (McGovern *et al*., 1998). Based on these previous studies, the presence of muscle bundles in ovaries of *E. morio*, *M. bonaci* and *M. microlepis* from the southern Gulf of Mexico can be considered an accurate criterion to distinguish between immature and regenerating females. Regenerating females with prominent muscle bundles in ovaries have been generally observed year round in these three grouper species. Therefore, use of this histological criterion to distinguish regenerating and immature females does not depend on sample collection timing relative to each species' spawning season. Notwithstanding, muscle bundle incidence was lower in the ovaries of females collected during peak spawning seasons and higher in the ovaries of females caught during nonreproductive seasons (Brulé *et al*., 1999, 2003a, 2003b). In other grouper species, the presence of muscle bundles in ovaries may be more seasonal. For example, muscle bundles become evident in the ovaries of *E. guttatus* following expansion and subsequent post-spawning collapse of ovarian lamellae. In addition, regenerating *E. guttatus* and *E. polyphekadion* females with muscle bundles in ovaries have only been observed from five to seven months after spawning (Shapiro *et al*., 1993; Sadovy *et al*., 1994; Rhodes and Sadovy, 2002). Reliable information on female reproductive status for these grouper species is therefore only available within a specific time period during the reproductive cycle. To avoid potential bias in maturity ogives and L_{50} estimates, maturity data must be collected only during this time period.

Year-round presence of muscle bundles is a possible indicator of the skipped spawning phenomenon (nonannual spawning; Rideout *et al*., 2005). In the present study, co-occurrence of muscle bundles with only PG oocyte stages in the ovaries of some *E. morio* and *M. bonaci* females caught during the spawning season suggests that annual spawning omission is occurring in these species (Brulé *et al*., 1999, 2003a).

Given the present results, a risk of misclassification between regenerating and immature females persists for some individuals in the studied species, even when using histological examination of ovaries. Comparison of the median FL and FL-frequency distribution between females in different reproductive phases indicates that uncertain maturity females are most probably immature (*E. morio*) or regenerating (*M. bonaci*), depending on species. In the case of *M. microlepis*, however, fish classified as uncertain maturity maintain a nebulous reproductive status, even when size is considered. The proportion of females classified as uncertain maturity differs between species, and is much higher in *E. morio* than in the other two species. The risk of misclassifying fish reproductive status is consequently higher for *E. morio* than for *M. bonaci* and *M. microlepis*, which could produce greater under- or overestimation of female L_{50} in *E. morio*. In these cases, it may be statistically prudent to exclude all females classified as uncertain maturity from the database to produce a reasonable approximation of L_{50}.

A final consideration is that, in some protogynous serranids, the females which undergo sexual transition may be misclassified as regenerating specimens. In most species, sex change occurs after the fish enters the regenerating phase. In the epinephelines *Epinephelus* and *Mycteroperca*, for instance, testicular tissue appears sporadically throughout the germinal epithelia of ovarian lamellae rather than forming in discrete islets (Sadovy de Mitcheson and Liu, 2008). Transitional specimens are characterized by the presence of PG oocytes and late-stage atresia intermixed with crypts of spermatogenetic cells (Shapiro *et al*., 1993). The latter are sometimes difficult to observe and identify. However, transitional specimens are known to be relatively uncommon in field collections (Sadovy and Shapiro, 1987), so their probable misclassification is unlikely to have a significant effect on the quality of maturity data.

Acknowledgments. – This research was funded by European Community (EC) grant C1*0432 ME(JR), the Science and Technology Council of the French Embassy in Mexico (CST) grant P 65000 ME, the Fondo Mexicano para la Conservación de la Naturaleza

(FMCN) grant C-1-99/062 and the Science and Technology Council of Mexico (CONACYT), grants 2184P-B9507 and 37606-B. Collections were made possible through the SEMARNAP/S.S.S. *24 de Febrero*/CINVESTAV agreement for use of the fishing vessel *UNICAP VII*, and authorized with fishing license No. 0330400-213-03 from SEMARNAP.

REFERENCES

BRULÉ T., DÉNIEL C., COLÁS-MARRUFO T. & SÁNCHEZ-CRESPO M., 1999. - Red grouper reproduction in the southern Gulf of Mexico. *Trans. Am. Fish. Soc.*, 128: 385-402.

BRULÉ T., RENÁN X., COLÁS-MARRUFO T., HAUYON Y., TUZ-SULUB A. & DÉNIEL C., 2003a. - Reproduction in the protogynous grouper *Mycteroperca bonaci* (Poey) from the southern Gulf of Mexico. *Fish. Bull.*, 101: 463-475.

BRULÉ T., DÉNIEL C., COLÁS-MARRUFO T. & RENÁN X., 2003b. - Reproductive biology of gag in the southern Gulf of Mexico. *J. Fish Biol.*, 63: 1505-1520.

BRULÉ T., NÓH-QUIÑONES V.E., SÁNCHEZ-CRESPO M., COLÁS-MARRUFO T. & PÉREZ-DÍAZ E., 2009. - Composición de las capturas comerciales del complejo Mero-pargo en el sureste del Golfo de México e implicaciones para el manejo de su pesquería. *Proc. Gulf Caribb. Fish. Inst.*, 61: 198-209.

BROWN-PETERSON N.J., WYANSKY D.M., SABORIDO-REY F., MACEWICZ B.J. & LOWERRE-BARBIERI S.K., 2011. - A Standardized terminology for describing reproductive development in fishes. *Mar. Coast. Fish.*, 3: 52-70.

BURGOS R. & DEFEO O., 2004. - Long-term population structure, mortality and modeling of a tropical multi-fleet fishery: the red grouper *Epinephelus morio* of the Campeche Bank, Gulf of Mexico. *Fish. Res.*, 66: 325-335.

CARTER J., MARROW G.J. & PRYOR V., 1994. - Aspects of the ecology and reproduction of Nassau grouper, *Epinephelus striatus*, off the coast of Belize, Central America. *Proc. Gulf Caribb. Fish. Inst.*, 43: 65-111.

COLÁS-MARRUFO T., BRULÉ T. & DÉNIEL C., 1998. - Análisis preliminar de las capturas de meros realizadas a través de unidades de la flota mayor en el sureste del Golfo de México. *Proc. Gulf Carib. Fish. Inst.*, 50: 780-803.

COLEMAN F.C., KOENIG C.C., HUNTSMAN G.R., MUSICK J.A., EKLUND A.M., MCGOVERN J.C., CHAPMAN R.W., SEDBERRY G.R. & GRIMES C.B., 2000. - Long-lived reef fishes: The grouper-snapper complex. *Fisheries*, 2 (3): 14-21.

DOMEIER M.L. & COLIN P.L., 1997. - Tropical reef fish spawning aggregations: defined and reviewed. *Bull. Mar. Sci.,* 60: 698-726.

GABE M., 1968. - Techniques histologiques. 1113 p. Paris: Masson.

HARRIS P.J., WYANSKI D.M., WHITE D.B. & MOORE J.L., 2002. - Age, growth, and reproduction of scamp, *Mycteroperca phenax*, in the southwestern North Atlantic, 1979-1997. *Bull. Mar. Sci.*, 70: 113-132.

ICES (INTERNATIONAL COUNCIL FOR EXPLORATION OF THE SEA), 2008. - Report of the workshop on maturity ogive estimation for stock assessment (WKMOG), 3-6 June 2008, Lisbon, Portugal. ICES cm2008/ACOM, 33, 72 p.

LEVIN P.S. & GRIMES C.B., 2002. - Reef fish ecology and grouper conservation and management. *In*: Coral reef fishes: dynamics and diversity in a complex ecosystem (Sale P., ed.), pp. 377-389. San Diego, CA: Elsevier Science.

LYON H., DUNCAN M., COLLINS A., COOK M., FITZHUGH G. & FIORAMONTI C., 2008. - Chapter 9: Histological classification for gonads of gonochoristic and hermaphroditic fishes. *In*: Procedural manual for age, growth and reproductive lab. 3rd edit. (Lombardi-Carlson L., Fioramonti C. & Cook M., eds), pp. 1-18, Panama City: Laboratory Contribution 08-15.

LOWERRE-BARBIERI S.K., HENDERSON N., LLOPIZ J., WALTERS S., BICKFORD J. & MULLER R., 2009. - Defining a spawning population (spotted seatrout *Cynoscion nebulosus*) over temporal, spatial, and demographic scales. *Mar. Ecol. Prog. Ser.,* 394: 231-245.

McGOVERN J.C., WYANSKI D.L., PASHUK O., MANOOCH II C.S. & SEDBERRY G.R., 1998. - Changes in the sex ratio and size at maturity of gag, *Mycteroperca microlepis*, from the Atlantic coast of the southeastern United States during 1976-1995. *Fish. Bull.*, 96: 797-807.

MORGAN M.J., 2008. - Integrating reproductive biology into scientific advice for fisheries management. *J. Northw. Atl. Fish. Sci.*, 41: 37-51.

RHODES K.L. & SADOVY Y., 2002. - Reproduction in the camouflage grouper (Pisces: Serranidae) in Pohnpei, Federate States of Micronesia. *Bull. Mar. Sci.*, 70: 851-869.

RIDEOUT R.M., ROSE G.A. & BURTON M.P.M., 2005. - Skipped spawning in female iteroparous fishes. *Fish. Fish.*, 6: 50-72.

SADOVY DE MITCHESON Y. & LIU M., 2008. - Functional hermaphroditism in teleosts. *Fish. Fish.*, 9: 1-43.

SADOVY Y. & SHAPIRO D.Y., 1987. - Criteria for the diagnosis of hermaphroditism in fishes. *Copeia*, 1987: 136-156.

SADOVY Y., ROSARIO A. & ROMÁN A., 1994. - Reproduction in an aggregating grouper, the red hind, *Epinephelus guttatus*. *Environ. Biol. Fish*, 41: 269-286.

SAGARPA (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación), 2012. - Carta nacional pesquera. *In*: Diario oficial de la federación, Tomo DCCVII, No 18, segunda sección (López-González A., ed.), pp. 21-128, Mexico City: Secretaría de Gobernación.

SCHERRER B., 1984. - Biostatistique. 850 p. Boucherville, Québec: Gaëtan Morin Editeur.

SHAPIRO D.Y., SADOVY Y. & MCGEHEE M.A., 1993. - Periodicity of sex change and reproduction in the red hind, *Epinephelus guttatus*, a protogynous grouper. *Bull. Mar. Sci.*, 53: 1151-1162.

VITALE F., SVEDÄNG H. & CARDINALE M., 2006. - Histological analysis invalidates macroscopically determined maturity ogives of the Kattegat cod (*Gadus morhua*) and suggests new proxies for estimating maturity status of individual fish. *ICES J. Mar. Sci.*, 63: 485-492.

WALLACE R.A. & SELMAN K., 1981. - Cellular and dynamic aspects of oocyte growth in Teleosts. *Am. Zool.*, 21: 325-343.